

7-Dehydrobrefeldin A, a Naturally Occurring Brefeldin A Derivative, Inhibits Secretion and Causes a *cis*-to-*trans* Breakdown of Golgi Stacks in Plant Cells¹

Azeddine Driouich, Alain Jauneau, and L. Andrew Staehelin*

Centre National de la Recherche Scientifique Unité de Recherche Associée 203, Université de Rouen, 76821 Mont Saint Aignan Cedex, France (A.D., A.J.); and Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347 (L.A.S.)

7-Dehydrobrefeldin A (7-oxo-BFA) is a brefeldin A (BFA) analog that, like BFA, is a potent phytotoxin of *Alternaria carthami*, a fungal pathogen of safflower (*Carthamus tinctorius* L.) plants. Both BFA and 7-oxo-BFA have been shown to be causal agents of the leaf spot disease of these plants. We have investigated the effects of 7-oxo-BFA on the secretion and the structure of the Golgi stacks of sycamore maple (*Acer pseudoplatanus*) suspension-cultured cells to determine whether 7-oxo-BFA affects these cells in the same manner as BFA. When applied at 10 $\mu\text{g}/\text{mL}$ for 1 h, 7-oxo-BFA inhibits secretion of proteins by approximately 80%, the same value obtained for BFA. However, electron micrographs of high-pressure frozen/freez-substituted cells demonstrated that 7-oxo-BFA is a more potent disrupter of the Golgi stacks of sycamore maple cells than BFA. In cells treated for 1 h with 10 $\mu\text{g}/\text{mL}$ 7-oxo-BFA, very few Golgi stacks can be discerned. Most of those that are left consist of fewer than three cisternae, all of which stain like *trans*-Golgi cisternae. They are surrounded by clusters of large (150–300 nm in diameter), darkly staining vesicles that are embedded in a fine-filamentous, ribosome-excluding matrix. Similarly sized and stained vesicles are seen budding from the rims of the residual *trans*-Golgi cisternae. Both the large vesicles and the residual Golgi stack buds stain with anti-xyloglucan polysaccharide antibodies. Recovery of Golgi stacks after removal of 7-oxo-BFA from 1-h-treated cells takes 2 to 6 h, compared with 1 to 2 h for cells treated with BFA. In contrast to 7-oxo-BFA, the BFA breakdown product BFA acid had no effect either on secretion or on the secretory apparatus. This is the first report, to our knowledge, of a BFA analog inhibiting secretion in a eukaryotic cell system.

The fungal toxin BFA has been used extensively by cell biologists to investigate membrane trafficking in the secretory pathway of both animal and plant cells (Klausner et al., 1992; Satiat-Jeunemaitre and Hawes, 1994). In most mammalian cells, 1 to 10 $\mu\text{g}/\text{mL}$ BFA not only inhibits secretion (Misumi et al., 1986; Fujiwara et al., 1988) but also causes profound morphological changes, including the disintegration of the Golgi apparatus and the redistribution of Golgi enzymes to the ER (Doms et al., 1989; Lippincott-

Schwartz et al., 1989, 1990; Orci et al., 1991; Pelham, 1991). All of these effects have been traced to BFA's inhibition of the guanine nucleotide-exchange reaction on the ADP-ribosylation factor, a small GTP-binding protein that regulates the assembly of coatamer proteins and clathrin coats on budding ER, Golgi, and *trans*-Golgi network vesicles (Donaldson et al., 1992; Helms and Rothman, 1992; Stamnes and Rothman, 1993; Staehelin and Moore, 1995). In the absence of these coats, the budding vesicles do not form properly and some convert into tubes, which, apparently due to the presence of specific targeting and fusion molecules, have the ability to fuse specifically with other membrane systems. This fusion leads to the rapid redistribution of Golgi enzymes into the ER. Remarkably, all of these changes are reversible upon removal of the drug.

In plants, BFA has also been shown to alter the structure of the Golgi apparatus and to inhibit secretion, but the responses differ in many respects from what has been reported for mammalian cells. Satiat-Jeunemaitre and Hawes (1992a, 1992b, 1993) were the first to demonstrate that treatment of maize and onion roots and maize and carrot suspension-cultured cells with 50 to 200 $\mu\text{g}/\text{mL}$ BFA induces a reversible vesiculation and disassembly of Golgi stacks. However, based on staining with the Golgi-specific antibody JIM84, no evidence of fusion of the Golgi-derived vesicles with ER membranes was obtained. In our BFA studies with sycamore (*Acer pseudoplatanus*) suspension-cultured cells (Driouich et al., 1993; A. Driouich, unpublished data), we found that, when applied at concentrations comparable to those used in mammalian cell studies (2.5–10 $\mu\text{g}/\text{mL}$), BFA was able to inhibit secretion and modify the glycosylation patterns of glycoproteins and polysaccharides in the absence of any morphological signs of breakdown of the Golgi stacks. Instead, we observed an increase in the number of *trans*-like Golgi cisternae and the accumulation of many *trans*-Golgi-derived vesicles in the adjacent cytoplasm. At 50 $\mu\text{g}/\text{mL}$ BFA, however, the sycamore maple Golgi stacks also vesiculate (A. Driouich, unpublished results). Kimura et al. (1993) and Schindler et al. (1994) also reported that low (10–20 $\mu\text{g}/\text{mL}$) concentrations of BFA can disrupt secretory functions in rice and

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* Corresponding author; e-mail staeheli@spot.colorado.edu; fax 1-303-492-7744.

Abbreviations: BFA, brefeldin A; EM, electron microscope; 7-oxo-BFA, 7-dehydrobrefeldin A.

maize cells, respectively, without causing a loss of Golgi stacks. Similar concentrations of BFA have also been shown to disrupt transport between the *trans*-Golgi network and vacuoles (Gomez and Chrispeels, 1993; Holwerda and Rogers, 1993), but no information concerning the state of the Golgi stacks was provided in those reports. In contrast, even 1 $\mu\text{g}/\text{mL}$ BFA is sufficient to inhibit both germination and growth of tobacco pollen tubes and also cause a breakdown of Golgi stacks in these cells (Rutten and Knuiman, 1993). Finally, in experiments with tobacco BY-2 suspension-cultured cells, it was found that ER-to-Golgi transport could be inhibited by 3.3 $\mu\text{g}/\text{mL}$ BFA (Matsuoka et al., 1995), whereas 20 μM BFA applied for 15 to 60 min to dividing cells could induce occasionally the fusion of some ER-like cisternae to Golgi cisternae, with minor effects on Golgi stack morphology (Yasuhara et al., 1995). Most of the changes involved an apparent decrease in the number of stacks and a decrease in their diameter. Stacks of hybrid ER-Golgi membranes have also been observed in tobacco protoplasts exposed to 10 $\mu\text{g}/\text{mL}$ BFA for 3 h (Kaneko et al., 1994). Taken together, these investigations demonstrate that (a) the BFA-induced inhibition of secretion does not require the disintegration of Golgi stacks, (b) the sensitivity of Golgi stacks to BFA differs significantly between cell types, (c) the disintegration of Golgi stacks is coupled to the vesiculation of the cisternae but rarely involves fusion of Golgi cisternae with ER membranes, and (d) when fusion does occur, the resulting ER-Golgi hybrid cisternae retain a unique type of stacked membrane configuration.

In this paper we report the effects of two BFA analogs on the secretory apparatus of sycamore maple suspension-cultured cells. In mammalian cells, none of the BFA analogs tested to date (7-oxo-BFA, 4,7-dioxo-BFA, BFA acid, and 7-epi and 4,7-diacetyl-10-epoxy-derivatives of BFA) has exhibited any effect on Golgi morphology, the processing of secretory and membrane molecules, or secretion (Orci et al., 1991; Brüning et al., 1992; Donaldson et al., 1992; Klausner et al., 1992). However, in safflower (*Carthamus tinctorius*) both BFA and 7-oxo-BFA (Fig. 1) have been shown to produce the same disease symptoms (i.e. brown necrotic spots on leaves) observed after infection with the fungus *Alternaria carthami*, which produces both of these phytotoxins (Tietjen et al., 1983). BFA is also known to inhibit the production and accumulation of phytoalexins by suspension-cultured safflower cells, consistent with the idea that this toxin interferes with the defense mechanisms of safflower plants (Tietjen and Matern, 1984). In light of

these findings, we wanted to determine whether BFA and 7-oxo-BFA exhibited similar effects on the secretory apparatus of sycamore maple suspension-cultured cells or whether 7-oxo-BFA exerted its toxic effects through a different pathway. We report that, like BFA, 7-oxo-BFA interferes with the secretory pathway of plant cells, but in sycamore maple suspension-cultured cells it is a more potent disrupter of Golgi stacks. As a control system we have used another BFA derivative, BFA acid (Fig. 1), which is a natural breakdown product of BFA. BFA acid can be produced by a one-step hydrolysis of the macrolide ester bond of BFA by an esterase from the bacterium *Bacillus subtilis* and has no known toxic effects on safflower plants (Kneusel et al., 1990). In our experiments BFA acid also exhibited no effects on either secretion or Golgi stack morphology.

MATERIALS AND METHODS

Sycamore (*Acer pseudoplatanus*) suspension cells were grown in a modified M6 medium (Torrey and Shigemura, 1957) on a shaker at 25°C in the dark and harvested for treatment at the log phase of growth, as described by Zhang and Staehelin (1992).

Radiolabeling Experiments and Drug Treatments

Cells were harvested and filtered and then washed and resuspended in fresh culture medium lacking Suc (3 g cells/10 mL), as described by Driouich et al. (1989, 1992). Radioactive labeling was done for 60 min with [^{35}S]Met in the presence or absence of 10 $\mu\text{g}/\text{mL}$ 7-oxo-BFA or BFA acid. The drugs were present at the same concentration during the labeling period.

After the cells were labeled and treated, the medium was collected by filtration and the cells were incubated again with fresh culture medium containing 1 M NaCl to extract the noncovalently/ionically bound cell wall proteins, as previously described (Driouich et al., 1989). Secreted proteins (culture medium plus NaCl-extracted proteins) and intracellular proteins were precipitated, processed, and used to measure the incorporated radioactivity, as described by Driouich et al. (1989, 1993).

Electron Microscopy

After treatment with 7-oxo-BFA or BFA acid (10 $\mu\text{g}/\text{mL}$ final concentration) for 60 min, sycamore cells (except those used for the recovery experiments) were fixed by high-pressure freezing and then freeze-substituted, infiltrated with resin, and embedded for EM analysis, as described by Zhang and Staehelin (1992). After BFA treatment and recovery, cells in the recovery experiments were chemically fixed and processed for EM analysis as described by Jauneau et al. (1992). Thin sections were viewed and photographed on a transmission EM (model CM-10, Philips, Eindhoven, The Netherlands, or model 109, Zeiss). Immunolabeling with anti-xyloglucan antibodies was also performed as described previously (Zhang and Staehelin, 1992).

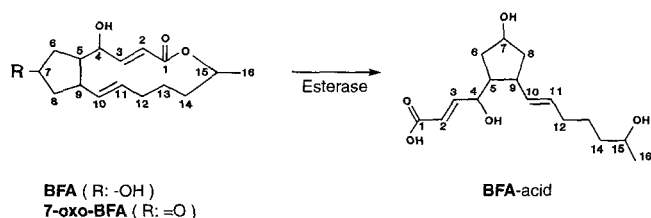


Figure 1. Chemical structure of BFA, 7-oxo-BFA, and BFA acid. BFA and 7-oxo-BFA can be isolated from *A. carthami* cultures, and BFA acid is produced by the hydrolysis of BFA with an esterase from the bacterium *B. subtilis* BG3.

RESULTS

7-Oxo-BFA but Not BFA Acid Inhibits Protein Secretion

To investigate the effect of 7-oxo-BFA on the synthesis and secretion of proteins, sycamore maple suspension-cultured cells were labeled with [35 S]Met for 60 min in the presence or absence of the drug (10 μ g/mL), and the incorporation of the label into intracellular and secreted proteins was analyzed. As shown in Figure 2, 7-oxo-BFA had almost no effect on the incorporation of [35 S]Met into cellular proteins but inhibited protein secretion by approximately 82%. Thus, the large inhibition of protein secretion by 7-oxo-BFA was not due to a general decrease in protein biosynthesis but instead was caused by an effect on the secretory apparatus. In contrast, BFA acid had only a minor effect on the synthesis and secretion of proteins (Fig. 2).

7-Oxo-BFA-Induced Disruption of Golgi Stacks Affects *cis*-, Medial, and *trans*-Cisternae Differentially

To determine whether the inhibition of secretion by 7-oxo-BFA is brought about by the disruption of the Golgi apparatus or by some other means, the treated cells were high-pressure frozen and analyzed by thin-section electron microscopy. As illustrated in Figure 3, sycamore maple suspension-cultured cells preserved by high-pressure freezing typically exhibited Golgi stacks consisting of five to six cisternae, which can be subdivided into *cis*, medial, and *trans* types based on morphological criteria (Staehelin et al., 1990; Zhang and Staehelin, 1992). After the cells were exposed to 10 μ g/mL 7-oxo-BFA for 60 min, very few Golgi stacks could be discerned in the cytoplasm (Figs. 4 and 5). Most of those left consisted of fewer than three cisternae and were surrounded by clusters of large, darkly staining

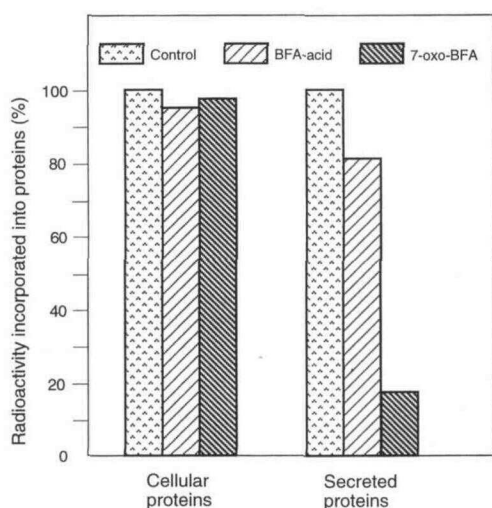


Figure 2. Effect of 7-oxo-BFA acid on the synthesis and secretion of proteins in sycamore suspension-cultured cells. Cells were radiolabeled with [35 S]Met in the presence or absence of 10 μ g/mL 7-oxo-BFA or BFA acid for 60 min, and the percentage of the radiolabel incorporated into intracellular and secreted proteins was determined (see "Materials and Methods"). The results are means of two separate experiments.

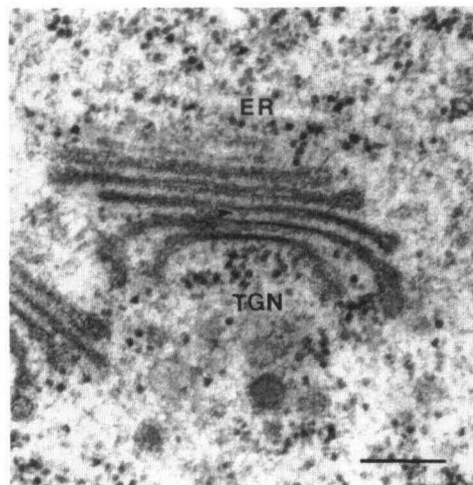


Figure 3. Thin-section electron microscopy of a Golgi stack of a high-pressure frozen/freeze-substituted sycamore maple suspension-cultured cell. The *cis* (top cisterna), medial (next two cisternae), and *trans*-Golgi cisternae (bottom three cisternae) can be distinguished based on their typical staining patterns. Note in particular the darkly staining, collapsed luminal contents of the *trans*-cisternae, which are also evident in the residual Golgi stacks shown in Figures 4B and 5A. TGN, *trans*-Golgi network; arrowhead, intercisternal elements. Bar, 0.2 μ m.

vesicles embedded in a fine-filamentous, ribosome-excluding matrix. Closer examination of the residual Golgi stacks revealed that the remaining cisternae were of the *trans* type, as evidenced by the collapsed, densely stained luminal space and the presence of intercisternal elements (Figs. 4B and 5; Staehelin et al., 1990). Typical of these residual cisternae were their bulbous margins, which were much larger than those of control Golgi stacks (150–300 versus 50–80 nm in diameter) and filled with materials that exhibited staining patterns similar to the contents of the same size vesicles in the adjacent cytoplasm (compare Fig. 3 with Figs. 4 and 5). These similarities suggest that the large cytoplasmic vesicles arise from a budding process from the residual *trans*-Golgi cisternae, an idea supported by the fact that both the Golgi and the cytoplasmic vesicles could be labeled with anti-xyloglucan antibodies (Fig. 5B).

Xyloglucan is a cell wall matrix polysaccharide, the synthesis of which has been localized to *trans*-Golgi cisternae in sycamore suspension-cultured cells (Zhang and Staehelin, 1992). Many of the remaining cisternae also displayed blunt-ended margins (Fig. 5), which would be expected if the large vesicles detached by simply pulling away from the cisternae, as is suggested by the image of a large vesicle seemingly breaking away from the bottom cisterna in Figure 5A. Upon removal of 7-oxo-BFA from the growth medium, cells took between 2 and 6 h to reassemble a full complement of normal-looking Golgi stacks (Fig. 6A).

In contrast to 7-oxo-BFA, BFA acid applied to sycamore maple suspension-cultured cells at a concentration of 10 μ g/mL for 1 h had no major effect on the morphology of the Golgi stacks (Fig. 6B), consistent with its minimal effect on the rate of protein secretion illustrated in Figure 2. Even when the BFA acid concentration was increased to 50 μ g/

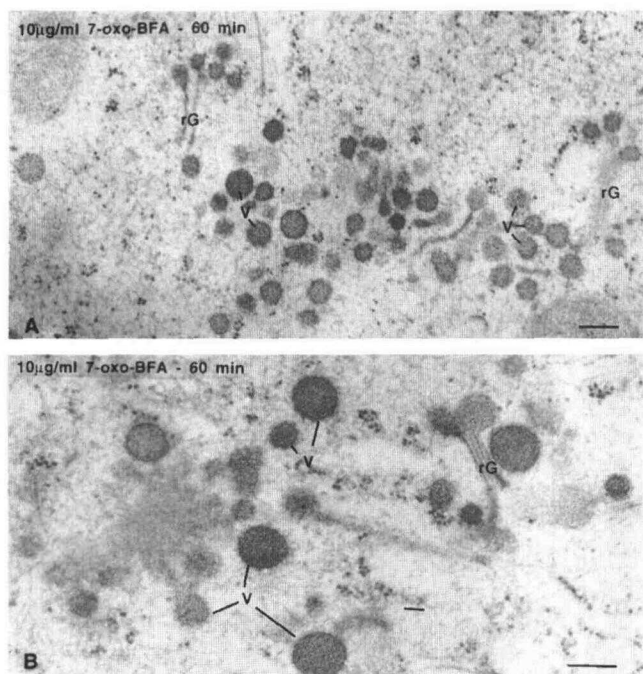


Figure 4. Electron micrographs of residual Golgi stacks (rG) and *trans*-Golgi-derived vesicles (V) in sycamore maple suspension-cultured cells treated with 10 $\mu\text{g/mL}$ 7-oxo-BFA for 60 min. A, Overview of a cytoplasmic domain containing characteristic large, darkly staining vesicles and several residual Golgi cisternae and stacks. B, Higher magnification view of vesicles and a residual Golgi stack. Bar, 0.2 μm .

mL, the Golgi stacks retained a normal appearance (data not shown).

DISCUSSION

Plant and Animal Golgi Stacks Respond Differently to 7-Oxo-BFA

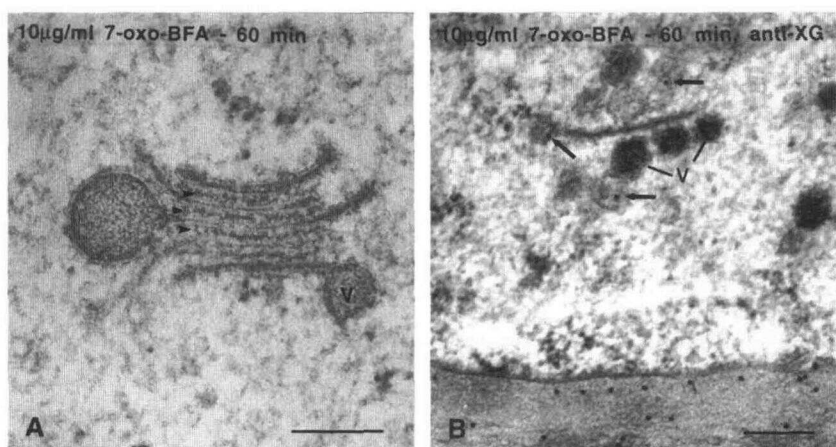
The central finding of this study was that the naturally occurring BFA analog, 7-oxo-BFA, applied at a concentration of 10 $\mu\text{g/mL}$ for 1 h to sycamore maple suspension-cultured cells both inhibited secretion and caused a breakdown of Golgi stacks. In contrast, another BFA analog, BFA

acid, had little effect on either secretion or Golgi stack morphology. These results are of interest to both plant and animal cell researchers, but for different reasons. Although the Golgi apparatuses of plant and animal cells perform many identical functions and have a similar basic design, they also differ in many important respects. Common to both is the basic architecture of the Golgi stacks and their ability to process *N*-linked glycans. Unique features of the plant Golgi apparatus include the number of discrete Golgi stack-*trans*-Golgi network units per cell (usually several hundred), their spatial organization (dispersed with no specific relationship to ER cisternae), their functional activity during cytokinesis, and their ability to synthesize complex polysaccharides without input from the ER.

In studies of mammalian cells, none of the BFA analogs examined to date, including 7-oxo-BFA, has been found to disrupt secretion and Golgi morphology like BFA (Brüning et al., 1992; Klausner et al., 1992). Thus, to our knowledge, this is the first demonstration that a BFA analog can mimic the action of BFA on the secretory apparatus of a eukaryotic cell. Nevertheless, the demonstration that 7-oxo-BFA can block protein secretion and cause a breakdown of Golgi stacks in sycamore maple cells is not totally unexpected, considering that both BFA and 7-oxo-BFA have been shown to produce the same disease symptoms in safflower leaves as infections by the fungus *A. carthami*, which is capable of producing both of these toxins (Tietjen et al., 1983; Tietjen and Matern, 1984). Thus, this study provides strong support for the hypothesis that both BFA and 7-oxo-BFA exert their toxic effects on plant cells by disrupting the functions of the secretory pathway.

The lack of effect of BFA acid on the structure and function of the Golgi apparatus of sycamore maple cells, even at a concentration of 50 $\mu\text{g/mL}$, is consistent with the finding that this BFA breakdown product is not toxic to safflower plants (Kneusel et al., 1990). It also confirms that the ring structure of BFA and 7-oxo-BFA is critical both for their toxicity and for their effects on the secretory apparatus. It is interesting that transgenic safflower plants expressing a bacterial esterase capable of breaking down BFA and 7-oxo-BFA also show enhanced resistance to the fungus *A. carthami* (Kneusel et al., 1994).

Figure 5. Higher magnification views of a residual Golgi stack (A) and of dense vesicles (V) and a residual cisterna stained with anti-xyloglucan antibodies (B, arrows). Note the *trans*-type appearance of the residual Golgi stack cisternae and the presence of intercisternal elements (arrowheads) between the cisternae in A. Two large vesicles are also seen budding from the cisternal rims, the lower one of which seems to be in the process of breaking away from the cisterna. Bar, 0.2 μm .



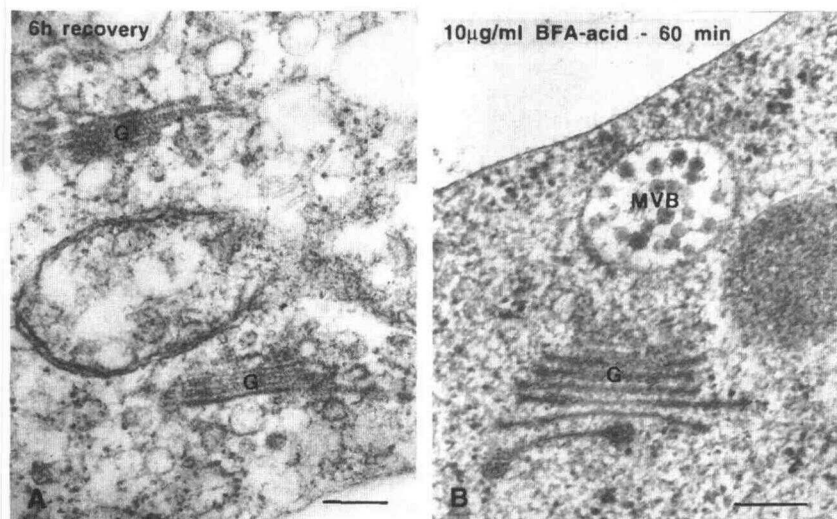


Figure 6. A, Two Golgi stacks (G) of a sycamore maple suspension-cultured cell allowed to recover for 6 h from a 1-h treatment with 7-oxo-BFA. Note that this is a micrograph of a chemically fixed cell. The Golgi exhibit a normal morphology. B, Electron microscopy of a Golgi stack (G) and multivesicular body (MVB) of a sycamore maple suspension-cultured cell treated for 1 h with 10 $\mu\text{g}/\text{mL}$ BFA acid. The stack exhibits no sign of breakdown. Bar, 0.2 μm .

7-Oxo-BFA Is a More Potent Disrupter of Plant Golgi Stacks than BFA

As discussed in the introduction, the sensitivity of different types of plant cells and plant species to externally applied BFA varies greatly, and inhibition of secretion can occur in the absence of Golgi stack disruption. This makes it difficult to compare the responses of different systems to BFA in quantitative terms. In this investigation we have circumvented some of these problems by using the same experimental system, sycamore maple suspension-cultured cells, and the same experimental protocols as in our previous BFA study (Driouich et al., 1993). In that study we observed that, over a 60-min period, 7.5 $\mu\text{g}/\text{mL}$ BFA reduced secretion by approximately 80% without causing any breakdown of the Golgi stacks. Since then we have repeated the experiment with 10 $\mu\text{g}/\text{mL}$ BFA and produced the same results (A. Driouich, unpublished results). Here we report that 10 $\mu\text{g}/\text{mL}$ 7-oxo-BFA applied for 1 h not only inhibits secretion by approximately 80% (Fig. 2) but also causes most of the Golgi stacks to break down into vesicles (Figs. 4 and 5). Furthermore, we have observed that recovery of the Golgi stacks from a 1-h treatment with 10 $\mu\text{g}/\text{mL}$ 7-oxo-BFA takes from 2 to 6 h (Fig. 6A), whereas only 1 to 2 h are needed for recovery after an equivalent experiment with BFA (Driouich et al., 1993). Taken together, these findings indicate that 7-oxo-BFA is a more potent disrupter of the Golgi stacks of sycamore maple suspension-cultured cells than BFA. Further studies are needed to determine why 7-oxo-BFA is a more potent disrupter of plant Golgi stacks than BFA and why the secretory apparatus of plant but not mammalian cells is sensitive to 7-oxo-BFA.

One possible explanation for the greater potency of 7-oxo-BFA for causing a breakdown of Golgi stacks is that this BFA analog might bind more tightly than BFA to a molecule involved in ER-to-Golgi vesicular transport. If this were the case, then 7-oxo-BFA could reduce the input of new membranes from the ER to *cis*-Golgi cisternae below the rate needed to compensate for membrane lost at the *trans* side of the stack due to the continued synthesis and

packaging of hemicellulosic secretory products by the *trans*-cisternae (Fig. 5; Zhang and Staehelin, 1992; Driouich et al., 1993). If intra-Golgi-stack transport continues, this imbalance would lead to a gradual loss of Golgi cisternae in a *cis*-to-*trans* direction. The reported vesiculation of Golgi stacks at >20 $\mu\text{g}/\text{mL}$ BFA (Satiat-Jeunemaitre and Hawes, 1994) may also result from inhibition of the same vesicle transport site. Testing of these ideas will require both the identification of the molecules that bind BFA and 7-oxo-BFA, respectively, and the exact localization of the site of action of these molecules.

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LITERATURE CITED

- Brüning A, Karrenbauer A, Schnabel E, Wieland F (1992) Brefeldin A induced increase of sphingomyelin synthesis. *J Biol Chem* 267: 5052–5057
- Doms R, Russ G, Yewdell J (1989) Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum. *J Cell Biol* 109: 61–72
- Donaldson J, Finazzi D, Klausner R (1992) Brefeldin A inhibits Golgi membrane catalysed exchange of guanine nucleotide onto ARF protein. *Nature* 360: 350–352
- Driouich A, Gonnet P, Makkie M, Lainé A-C, Faye L (1989) The role of high-mannose and complex asparagine-linked glycans in the secretion and stability of glycoproteins. *Planta* 180: 96–104
- Driouich A, Lainé A-C, Vian B, Faye L (1992) Characterization and localization of laccase forms in stem and cell culture of sycamore. *Plant J* 2: 13–24
- Driouich A, Zhang GF, Staehelin LA (1993) Effect of brefeldin A on the structure of the Golgi apparatus and on the synthesis and secretion of proteins and polysaccharides in sycamore maple

- (*Acer pseudoplatanus*) suspension-cultured cells. *Plant Physiol* 101: 1363–1373
- Fujiwara T, Oda K, Yokota S, Takatsuki A, Ikehara Y (1988) Brefeldin A causes a disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. *J Biol Chem* 263: 18545–18552
- Gomez L, Chrispeels MJ (1993) Tonoplast and soluble vacuolar proteins are targeted by different mechanisms. *Plant Cell* 5: 1113–1124
- Helms J, Rothman J (1992) Inhibition by brefeldin A of a Golgi membrane enzyme that catalyzes exchange of guanine nucleotide bound to ARF. *Nature* 360: 352–354
- Holwerda BC, Rogers JC (1993) Structure, functional properties and vacuolar targeting of the barley thiol protease, aleurain (supplement). *J Exp Bot* 44: 321–339
- Jauneau A, Morvan C, Lefebvre F, Demarty M, Ripoll C, Thellier M (1992) Differential extractability of calcium and pectic substances in different wall regions of epicotyl cells in young flax plants. *J Histochem Cytochem* 40: 1183–1189
- Kaneko TS, Watanabe R, Sato M, Osumi M, Takatsuki A (1994) Morphological changes in Golgi apparatus induced by brefeldin A treatment in tobacco protoplasts. *Plant Morphol* 6: 1–11
- Kimura S, Yamada M, Igaue I, Mitsui T (1993) Structure and function of the Golgi complex in rice cells: characterization of Golgi membrane glycoproteins. *Plant Cell Physiol* 34: 855–863
- Klausner RD, Donaldson JG, Lippincott-Schwartz J (1992) Brefeldin A: insights into the control of membrane traffic and organelle structure. *J Cell Biol* 116: 1071–1080
- Kneusel RE, Matern U, Wray V, Kloppel KD (1990) Detoxification of the macrolide toxin brefeldin A by *Bacillus subtilis*. *FEBS Lett* 275: 107–110
- Kneusel RE, Schiltz E, Matern U (1994) Molecular characterization and cloning of an esterase which inactivates the macrolide toxin brefeldin A. *J Biol Chem* 269: 3449–3456
- Lippincott-Schwartz J, Donaldson JG, Schweizer A, Berger EG, Hauri HP, Yuan LC, Klausner RD (1990) Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. *Cell* 60: 821–836
- Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD (1989) Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* 56: 801–813
- Matsuoka K, Bassham DC, Raikhei NV, Nakamura K (1995) Different sensitivity to wortmannin of two vacuolar sorting signals indicates the presence of distinct sorting machineries in tobacco cells. *J Cell Biol* 130: 1307–1318
- Misumi Y, Micki A, Takatsuki A, Tamura G, Ikehara Y (1986) Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J Biol Chem* 261: 11398–11403
- Orci L, Tagaya M, Amherdt M, Perrelet A, Donaldson JG, Lippincott-Schwartz J, Klausner RD, Rothman JE (1991) Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. *Cell* 64: 1183–1195
- Pelham HRB (1991) Multiple targets for brefeldin A. *Cell* 67: 449–451
- Rutten TL, Knuiman B (1993) Brefeldin A effects on tobacco pollen tubes. *Eur J Cell Biol* 61: 247–255
- Satiat-Jeunemaitre B, Hawes C (1992a) Redistribution of a Golgi glycoprotein in plant cells treated with brefeldin A. *J Cell Sci* 103: 1153–1166
- Satiat-Jeunemaitre B, Hawes C (1992b) Reversible dissociation of the plant Golgi apparatus by brefeldin A. *Biol Cell* 74: 325–328
- Satiat-Jeunemaitre B, Hawes C (1993) Insights into the secretory pathway and vesicular transport in plant cells. *Biol Cell* 79: 7–15
- Satiat-Jeunemaitre B, Hawes C (1994) G.A.T.T. (a general agreement on traffic and transport) and brefeldin A in plant cells. *Plant Cell* 6: 463–467
- Schindler T, Bergfeld F, Hohl M, Schopfer P (1994) Inhibition of Golgi-apparatus function by brefeldin A in maize coleoptile and its consequences on auxin-mediated growth, cell-wall extensibility and secretion of cell-wall proteins. *Planta* 192: 404–413
- Staehelin LA, Giddings TH Jr, Kiss JZ, Sack FD (1990) Macromolecular differentiation of Golgi stacks in root tips of *Arabidopsis* and *Nicotiana* seedlings as visualized in high pressure frozen and freeze-substituted samples. *Protoplasma* 157: 75–91
- Staehelin LA, Moore I (1995) The plant Golgi apparatus: structure, functional organization and trafficking mechanisms. *Annu Rev Plant Physiol Plant Mol Biol* 46: 261–288
- Stammes MA, Rothman JE (1993) Binding of AP-1 clathrin adaptor particles to Golgi membranes requires ADP-ribosylation factor, a small GTP binding protein. *Cell* 73: 999–1005
- Tietjen KG, Matern U (1984) Induction and suppression of phytoalexin biosynthesis in cultured cells of safflower, *Carthamus tinctorius* L., by metabolites of *Alternaria carthami* Chowdhury. *Arch Biochem Biophys* 229: 136–144
- Tietjen KG, Schallert E, Matern U (1983) Phytotoxins from *Alternaria carthami* Chowdhury: structural identification and physiological significance. *Physiol Plant Pathol* 23: 387–400
- Torrey JG, Shigemura Y (1957) Growth and controlled morphogenesis in pea root callus tissue grown in liquid media. *Am J Bot* 44: 334–344
- Yasuhara H, Sonobe S, Shibaoka H (1995) Effects of brefeldin A on the formation of the cell plate in tobacco BY-2 cells. *Eur J Cell Biol* 66: 274–281
- Zhang GF, Staehelin LA (1992) Functional compartmentalization of the Golgi apparatus of plant cells. An immunocytochemical analysis of high-pressure frozen- and freeze-substituted sycamore maple suspension culture cells. *Plant Physiol* 99: 1070–1083